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Novel Sequence Types of *Chlamydia pecorum* Infect Free-Ranging Alpine Ibex (*Capra ibex*) and Red Deer (*Cervus elaphus*) in Switzerland

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ABSTRACT: *Chlamydia pecorum*, a recognized pathogen of domesticated ruminants and koalas (*Phascolarctos cinereus*), has been recently reported in a broad range of other wildlife species including water buffalo (*Bubalus bubalis*), ibex (*Capra ibex*), chamois (*Rupicapra rupicapra*), red deer (*Cervus elaphus*), and birds. This identification raises questions as to whether cross-host transmission may be a factor in the epidemiology of infections in these species. To begin to address this question, we employed a *C. pecorum* species-specific multilocus sequence typing (MLST) scheme to characterize a small collection of *C. pecorum*-positive samples from wild, free-range ibex, a chamois, and a red deer from Grison, Switzerland, a canton where domesticated and wild ruminants graze in close proximity during the summer. Screening by PCR confirmed low to moderate levels of *Chlamydia pecorum* DNA in the eyes of healthy ibex ($n=4$) and in the deer fecal sample ($n=1$). The MLST analysis revealed three novel sequence types (STs; 88, 90, and 89) in these samples. On phylogenetic analysis, the ibex and deer sequences clustered by host species in their own well-supported clades and away from *C. pecorum* STs found in other hosts. Even though the analyzed sample size was small, the identification of unique *C. pecorum* STs infecting free-ranging Alpine ibex and red deer provides useful information for further *C. pecorum* epidemiologic studies.

Key words: *Chlamydia pecorum*, cross-host transmission, molecular epidemiology, wild ruminants.

Chlamydia pecorum is an obligate intracellular bacterial pathogen and an important cause of disease in wild and domesticated animals. In Australia, *C. pecorum* is recognized as a major pathogen of the iconic koala (*Phascolarctos cinereus*), causing diseases including blindness and infertility (Polkinghorne et al., 2013). In livestock, *C. pecorum* may result in unapparent subclinical infections or may cause diseases including encephalomyelitis and polyarthritis (Jelocnik et al., 2014). *Chlamydia pecorum* has recently been detected in several previously unrecognized wild hosts including water buffalo (*Bubalus bubalis*; Greco et al. 2008), Alpine ibex (*Capra ibex*; Holzwarth et al. 2011a), chamois (*Rupicapra r. Rupicapra*; Holzwarth et al. 2011b), red deer (*Cervus elaphus*; Regenscheit et al. 2012) and birds (Frutos et al. 2012).

Detection of the same chlamydial species infecting wild and domesticated hosts raises questions about the potential role of cross-host transmission in these infections. In recent *C. pecorum* molecular typing studies, we found that genetically identical strains can be found in association with disease in koalas and Australian domestic sheep (*Ovis aries*), indicating the potential for cross-host transmission (Jelocnik et al. 2013, 2014). Furthermore, we observed in multiple cases that a single host may shed genetically distinct strains from different anatomical sites and that some strains could be associated with disease (Jelocnik et al. 2014).

The study centered on the canton of Grisons, Switzerland, where cograzing of wildlife and domesticated ruminants is common and where *C. pecorum* was identified in ibex (Holzwarth et al. 2011a), chamois (Holzwarth et al. 2011b), and red deer (Regenscheit et al. 2012). Molecular typing of these *C. pecorum*-positive samples from both of these groups from the sympatric populations was not performed at the time and will be necessary to evaluate whether any "spill-over" or "spill-back" has occurred between the populations. Here,

we applied a *C. pecorum*, species-specific, multi-locus sequence typing (MLST) to a subset of five *C. pecorum* PCR-positive samples from three ibex and a red deer to investigate the phylogenetic relationships of *C. pecorum* strains detected in wild Swiss ruminants to those described in domesticated animals.

The initial collection (Table 1) targeted in this study consisted of a previously screened cohort of ocular swabs collected from six hunted ibex ($n=7$; both eyes from one animal; Holzwarth et al. 2011a) and a chamois ($n=1$; Holzwarth et al. 2011b) and a fecal sample from a hunted red deer (Regenscheit et al. 2012). The chamois had corneal lesions and blindness; the other animals had no clinical signs of disease. The DNA extracted from each sample was previously screened for family *Chlamydiaceae* using a 23S rRNA gene *Chlamydiaceae*-specific real-time quantitative PCR (qPCR) assay (Ehricht et al. 2006). Chlamydial species were determined using a 23S ArrayTube (AT) microarray assay (Borel et al. 2008).

To confirm the previous identification of *C. pecorum* in these samples, we used a species-specific *C. pecorum* qPCR screen which targets a 202-base pair segment of the *C. pecorum* 16S rRNA gene (Marsh et al. 2011). The results of this screen and the relative *C. pecorum* load in each sample are outlined in Table 1. It revealed agreement in 7/8 (88%) of the samples, although the detected infectious loads were low (4.2×10^3) or very low (1.2×10^1) compared to loads detected in previous studies in sheep (Jelocnik et al. 2014; Yang et al. 2014).

Chlamydia pecorum MLST was applied to a subset of four *C. pecorum*-positive ocular samples from three ibex and a fecal sample from a red deer as described by Jelocnik et al. (2013). Likely due to the low *C. pecorum* loads, we were unsuccessful in typing the remaining samples by MLST. The concatenation and sequence analyses of the Swiss ruminant *C. pecorum* house-keeping gene fragment sequences was performed using Geneious Pro v7.0 (Biomatters Limited, http://www.geneious.com/). GenBank submissions for each house-keeping gene sequence can be found at KJ885626–KJ885660 (http://www.ncbi.nlm.nih.gov/genbank/). This analysis revealed three novel *C. pecorum* sequence types (STs) (see Supplementary Material, Table 1S) including ST 88 found in the ocular swabs of 3/4 ibex (Gri/Ibex405/LE, Gri/Ibex427/LE, and RE samples), ST 90 found in a single ibex ocular swab (Gri/Ibex385/RE), and ST 89 found in the deer fecal sample (Gri/Deer370/Fec). Ibex ST 88 differed from ST 90 by one allele but from the deer ST 89 by four alleles.

To evaluate the phylogenetic relationships among the *C. pecorum* STs detected in the Swiss ruminants and their relationship to *C. pecorum* from other hosts, a mid-point rooted Bayesian phylogenetic tree was constructed from the concatenated house-keeping gene sequence data sets of previously typed *C. pecorum* samples. Characteristics of the 28 *C. pecorum* STs derived from a range of hosts used for phylogenetic analyses are outlined in Table 1S. The MLST sequences from other hosts were obtained from the *Chlamydiales* MLST website (http://pubmlst.org/chlamydiales/) (Jolley and Maiden 2010). Tree parameters included an HKY+I nucleotide substitution model with four Markov Chain Monte Carlo (MCMC) chains with a million generations, sampled every 1,000 generations, and with the first 10,000 trees discarded as burn-in. Posterior probabilities are displayed on the nodes.

The ibex and deer sequences clustered by host species and away from *C. pecorum* STs found in cattle (*Bos primigenius*), sheep, pigs (*Sus scrofa*), and koalas (Fig. 1). The ibex clade was well supported, sharing the most sequence differences to pig and red deer isolates (average 16 and 12 nucleotides, respectively). An average of eight nucleotides also distinguished these sequences from those found in the koala, sheep, and cow isolates. From

TABLE 1. Swiss wild ruminant samples and results of AT MicroArray and *Chlamydia pecorum*-specific quantitative PCR screen including the *C. pecorum* loads for each sample. Samples in boldface were used for further multi-locus sequence typing analyses (MLST) analyses.

Isolate ID	Host[a]	Geographic location	Clinical symptoms[b]	Sample type	AT MicroArray identification[c]	*C. pecorum*-specific screen[d]	*C. pecorum* load[e]
Gri/Ibex405/LE	Ibex	Grisons	NCS (hunted animal)	Eye swab	*C. pecorum*	Positive	1.5×10^3
Gri/Ibex458/LE	Ibex	Grisons	NCS (hunted animal)	Eye swab	*C. pecorum*	Positive	1.2×10^1
Gri/Ibex427/RE	Ibex	Grisons	NCS (hunted animal)	Eye swab	*C. pecorum*, *Chlamydophila abortus*	Positive	4.2×10^3
Gri/Ibex427/LE	Ibex	Grisons	NCS (hunted animal)	Eye swab	*C. pecorum*, *Ch. abortus*	Positive	2.1×10^3
Gri/Ibex385/RE	Ibex	Grisons	NCS (hunted animal)	Eye swab	*C. pecorum*, *Ch. abortus*	Positive	1.8×10^3
Gri/Deer370/Fec	Red Deer	Grisons	NCS (hunted animal)	Feces	*C. pecorum*	Positive	1.1×10^3
515 L[f]	Chamois	Grisons	Corneal lesions and blindness	Eye swab	*C. pecorum*	Positive	4.2×10^1
VS 286 L	Ibex	Valais	NCS (hunted animal)	Eye swab	*C. pecorum*, *Ch. abortus*	Negative	Nil

[a] Ibex = *Capra ibex*; red deer = *Cervus elaphus*; Chamois = *Rupicapra rupicapra*.

[b] NCS= no clinical signs.

[c] Samples positive for *Chlamydiaceae*.

[d] In duplicate.

[e] Mean copy No. of 202-base pair *C. pecorum* 16S ribosomal DNA PCR product per microliter of the swab sample–extracted DNA.

[f] Also positive for *Mycoplasma conjunctivae* by PCR.

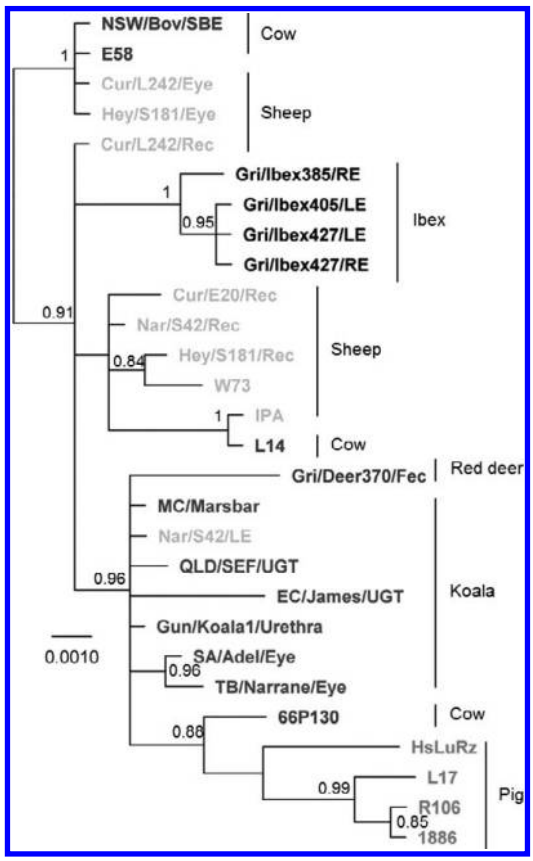

FIGURE 1. Mid-point rooted Bayesian phylogenetic analysis of concatenated sequences of seven house-keeping gene fragments of the *Chlamydia pecorum* isolates from three ibex (*Capra ibex*) and one red deer (*Cervus elaphus*). Previously described and concatenated *C. pecorum* house-keeping gene fragment sequences from four cattle (*Bos primigenius*), four domestic pigs (*Sus scrofa*), six koalas (*Phascolarctos cinereus*), and eight sheep (*Ovis aries*) were also included in the Bayesian phylogenetic analyses. Posterior probabilities of >0.80 are displayed on the tree nodes.

this very limited set of ibex *C. pecorum* STs, we observe that: a single host (Gri/Ibex 427) can shed identical *C. pecorum* STs from both eyes; the same STs can be detected in multiple hosts from a sympatric area (Gri/Ibex427/LE and RE and Gri/Ibex405/LE); and closely related, yet different STs can be found in one population. The deer *C. pecorum* ST 89 resolved in a larger diverse clade which consisted of koala, sheep, cattle, and pig subclades of *C. pecorum* STs, although in the same clade, *C. pecorum* Gri/Deer370/Fec ST 89 differed by an average of 8–16 nucleotides from the other STs analyzed.

Acknowledging the limited sample size, our most significant findings were the three novel *C. pecorum* STs infecting two wildlife host species. The great variability between ibex and deer *C. pecorum* strains isolated from sympatric animals is notable; however, it is not unusual for *C. pecorum*. In previous studies of *C. pecorum* strains from koala, sheep, and cows, we identified 17 novel *C. pecorum* STs, 11 from sheep and six from koalas, from a collection of 86 swab samples from Australian livestock and koalas (Jelocnik et al. 2013, 2014).

In the present study, *C. pecorum* presence in ocular swabs did not correlate with evidence of *Chlamydia*-related diseases. Assuming the almost ubiquitous presence of *Chlamydiae* in the gastrointestinal tract (Rank and Yeruva 2014), fecal shedding of *C. pecorum* in the red deer is consistent with observations in other ruminant species. Asymptomatic infections with chlamydiae, such as those observed in this study, have also been identified previously. Polkinghorne et al. (2009) identified chlamydial species colonizing the conjunctiva of otherwise healthy animals but could not link the identification to subsequent disease onset. In the animals in our current study, chamois 515 L was the only animal with evident pathology that was shedding very low levels of *C. pecorum* but had a diagnosed coinfection with *Mycoplasma conjunctivae* (Holzwarth et al. 2011b). Subsequently, *M. conjunctivae* was also associated with infectious keratoconjunctivitis in chamois (Mavrot et al. 2012).

The limited number of samples analyzed in this study makes drawing conclusions about cross-host transmission difficult. Nevertheless, the identification of unique *C. pecorum* STs infecting Swiss free-ranging ibex and red deer suggest that these strains are not being transmitted to the wider area outside Grisons, Switzerland. The identification of novel STs gives

us valuable insight into the host range for this animal pathogen and provides unique information for future epidemiologic studies of wildlife in this region and also for monitoring the epidemiology and impact of these infections on domesticated animals.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://doi:10.7589/2014-08-220.

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